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Identification of molecular targets in vulvar cancers

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HIGHLIGHTS

- We describe molecular alterations that contribute to vulvar cancer pathogenesis.
- Squamous cell carcinoma and adenocarcinoma of the vulva have differing molecular alterations.
- Specific drug targets of vulvar cancer are proposed.
- Molecularly-guided precision medicine could provide targeted treatment options.

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ABSTRACT

Objectives. To identify molecular alterations that contribute to vulvar cancer pathogenesis with the intent of identifying molecular targets for treatment.

Methods. After retrospective analysis of a database of molecularly-profiled gynecologic cancer patients, 149 vulvar cancer patients were included and tested centrally at a CLIA laboratory (Caris Life Sciences, Phoenix, AZ). Tests included one or more of the following: gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]), and gene amplification (C/FISH). A Fisher's exact test was used when indicated with a p-value ≤ 0.05 indicating significance.

Results. Median age was 65. 85% had squamous cell carcinoma (SCC) and 15% adenocarcinoma (ADC) histologies. 46% had metastatic (Stage IV) disease. Targeted hot-spot sequencing identified variants in the following genes: *TP53* (33%), *PIK3CA*/*BRCA2* (8%, 10%, respectively), *HRAS*/*FBXW7* (5%, 4%, respectively) and *ERBB4*/*GNAS* (3%, 3% respectively). Mutations in *AKT1*, *ATM*, *FGFR2*, *KRAS*, *NRAS* (n = 1, respectively) and *BRAF* (n = 2) also occurred. Specific protein changes for targetable genes included clinically pathogenic mutations commonly found in other cancers (e.g. *PIK3CA*: exon 9 [E545K], *RAS*: G13D, Q61L, *BRCA2*: S1667X, *BRAF*: R443T, *FBXW7*: E471fs, etc.). Drug targets identified by IHC and ISH methodologies include cMET (32% IHC, 2% ISH), PDL1 (18%), PTEN loss (56%), HER2 (4% IHC, 2% ISH) and hormone receptors (AR, 4%; ER, 11%; PR, 4%). Comparisons between SCC and ADC identified differential rates for AR, ER, HER2 and GNAS with an increased presence in ADC (p-values all <0.05).

Conclusions. Molecularly-guided precision medicine could provide vulvar cancer patients alternative, targeted treatment options.

1. Introduction

While rare, vulvar carcinoma carries a poor prognosis when advanced. There will be an estimated 6020 new cases in 2017, comprising only 0.4% of all new cancer cases; however, it is projected to cause approximately 1150 deaths in 2017. The incidence is on the rise, increasing 0.6% each year over the last 10 years [1]. Patients who present with regional spread (31%) and distant metastases (5%) have the worst prognosis, with a 5-year survival of 57% and 17%, respectively [1]. This is primarily due to limited therapeutic options. Patients with advanced vulvar carcinoma experience significantly shorter overall survival (OS) durations than those with other metastatic or recurrent solid tumors treated with novel phase I therapeutics [2].

Although 80–90% of vulvar carcinomas are squamous cell carcinomas (SCC), they remain clinically and pathologically heterogeneous. In contrast to cervical cancer, in which human papilloma virus (HPV) can be detected in 99.7% of cases, HPV is thought to be responsible for only 43–60% of vulvar SCC [3]. HPV oncoproteins E6 and E7 lead to the inactivation of tumor suppressor proteins p53 and retinoblastoma (Rb), respectively. These tumors are associated with diffuse expression

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of p16 [4,5]. The other half of vulvar SCC are considered "HPV independent" and commonly occur in older women, harbor *TP53* somatic mutations, and are associated with chronic inflammation such as lichen sclerosis [3,6]. Other genetic abnormalities described in HPV independent SCC include phosphatase and tensin homolog (*PTEN*) mutation and epidermal growth factor receptor (*EGFR*) activation [7,8]. Adenocarcinoma histology is a remarkably rare form of vulvar carcinoma, an already rare entity, resulting in it being even less studied than SCC of the vulva [9]. Given the rarity and heterogeneity of the disease, there is a limited understanding of the pathogenesis of vulvar cancer and even less knowledge available regarding targetable molecular pathways.

The National Comprehensive Cancer Network (NCCN) recently released guidelines for squamous vulvar cancers for the first time in 2016. Standard treatment for early disease is primarily surgical, including a wide radical excision with lymph node sampling. Advanced disease is often managed with adjuvant or neoadjuvant treatment consisting of radiation and/or chemotherapy [10]. The NCCN guidelines incorporate chemotherapies used for other HPV-induced cancers, including cisplatin, mitomycin-C, 5-fluorouracil, vinorelbine, and paclitaxel; however, given the paucity of data due to the rarity of this disease, targeted therapies are lacking from these recommendations. Chemotherapeutic agents that are active in other squamous cell cancers have proven to be less effective in vulvar carcinoma [11–13]. Some targeted therapies have been approved for other HPV-related SCC, including bevacizumab for advanced or recurrent cervical cancer and cetuximab for head and neck cancer. Immune checkpoint inhibitors have also shown promising results in head and neck SCC [14]. With this poor prognosis in advanced disease, the development of novel therapeutic regimens is warranted and necessary [14–16]. Molecular profiling is critical in further exploration of therapeutic options for these patients. Retrospectively, we examined a database of molecularly profiled patients for insight into the molecular alterations that contribute to vulvar pathogenesis with the hopes of identifying molecular targets for this rare disease.

2. Materials and methods

2.1. Patients and multiplatform molecular profiling

An institutional review board (IRB) approved, retrospective review of 149 cases of vulvar cancer submitted to a CLIA-certified laboratory for molecular profiling from 2010 to 2016 (Caris Life Sciences, Phoenix, AZ). H&E slides were reviewed by board-certified pathologist for sufficient tumor content, specimen quality and to verify the diagnosis on the pathology reports submitted with the tumor samples. Multi-platform molecular analysis on each specimen included at least one of the following: immunohistochemistry (IHC), in situ hybridization (ISH) and Sanger/next-generation sequencing (NGS). The testing performed for each patient varied based on the physician's request, tissue availability, technology advances (Sanger vs. NGS) and emerging clinical evidence for molecular biomarkers. Specimens utilized for molecular profiling were procured from patients with advanced/metastatic, recurrent, and/or refractory vulvar cancer. Other than age, demographic and clinical data were unavailable for analysis. A glossary of biomarker and gene acronyms is available in Supplementary Table S1. The term "altered" is used in the text and tables to indicate aberrant protein expression, gene copy number or gene mutations.

2.2. Immunohistochemistry

IHC analysis of 23 proteins was performed on formalin-fixed paraffin-embedded (FFPE) tumor samples using commercially available detection kits and automated staining techniques (Benchmark XT; Ventana Medical Systems, Tucson, AZ; and Autostainer- LInk 48; Dako, Carpinteria, CA). Antibody clones and thresholds used are provided in Supplementary Table S2. Appropriate positive and negative controls were used for all proteins tested. IHCs were scored manually by board-certified pathologists using a binary system of predefined thresholds consisting of intensity of staining (0, 1+, 2+, and 3+) and percentage of tumor cells that stained positive. Thresholds are derived from peer-reviewed clinical literature, which associates response to treatment to biomarker status. Tests are interpreted as positive or negative, and the expression data are represented as a distribution (percentage) of positive or negative results observed in the cohort tested.

2.3. In situ hybridization

Gene copy number alterations of *cMET*, *EGFR* and *HER2* were analyzed by DNA ISH using fluorescence in situ hybridization and/or chromogenic in situ hybridization probes as part of the automated staining techniques (Benchmark XT; Ventana Medical Systems) and automated imaging systems (BioView, Billerica, MA). Cutoffs are provided in the Supplementary Table S2. The ratio of gene to pericentromeric regions of chromosome 7 (*EGFR*, *cMET*) and 17 (*HER2*) were used to determine increases in gene copy number. Ratios higher than defined cutoff were considered positive and ratios less than defined cutoff were considered negative.

2.4. Sanger sequencing

Sanger sequencing included selected regions of *BRAF*, *cKIT*, *KRAS*, *NRAS*, and *PIK3CA* and was performed using M13-linked polymerase

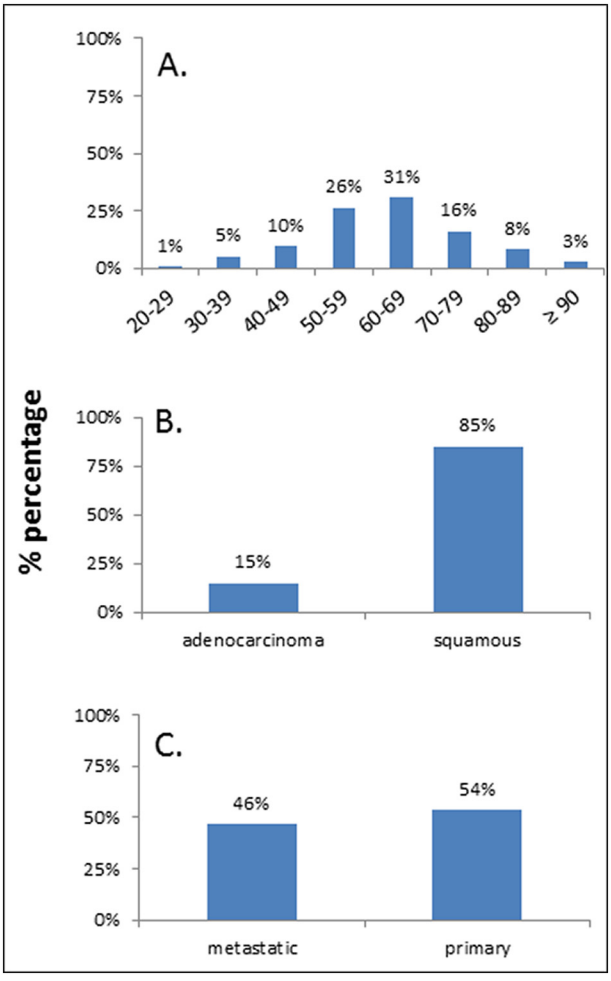

Fig. 1. Distribution of age (A), histology (B) and disease status (based on origin of specimen) (C) of vulvar patients included in this study.

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